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IDENTITY OF A CHLOROTIC RING-SPOT VIRUS FROM A KALE
By L. A. Alvarez-Garcia 115

A NEW DISEASE OF ORCHIDS IN PUERTO RICO; PYTHIUM BLACK ROT
By L. A. Alvarez-Garcia 126

THE FOOD OF *RANA CATESBEIANA* SHAW IN PUERTO RICO
By Mario E. Pérez 126

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IDENTITY OF A CHLOROTIC RING-SPOT VIRUS FROM KALE[1]

(*Brassica oleracea* var. *acephala*)

L. A. ALVAREZ-GARCÍA[2]

INTRODUCTION

A mosaic disease of kale (*Brassica oleracea* var. *acephala*) was found in vicinity of Ithaca, N. Y., by Dr. R. E. Wilkinson of the Cornell Plant Pathology Department, late in the fall of 1948. This mosaic is characterized by systemic mottling and chlorotic ring-spots on the leaves.

The occurrence of the disease in kale and perhaps in other crucifers in upper New York may be considered sporadic in nature and of no economic significance. The viral entity apparently is endemic in the State and has escaped recognition in cruciferous suscepts grown when the environment is not favorable for the multiplication of the virus, nor for its dissemination. There is the possibility, also, of its being a strain of low virulence, causing only mild symptoms on most crucifers.

Crucifer diseases due to viruses are considered, so far, of little economic importance in New York State. The first observation of mosaic in a crucifer was made by Stewart (15)[3] on cabbage (*B. oleracea* var. *capitata* L.), in 1910. The disease was characterized by necrotic flecks of the outer and inner head leaves. No mention was made of the nature of the disease.

Cauliflower (*B. oleraceae botrytis* L.) mosaic was first reported by Thatcher in 1927 (17). Clayton (2) described in 1930 a mosaic of rutabagas (*B. campestris* (L) *napobrassica* DC) which occurred commonly in the Port Jefferson section on Long Island. This mosaic has never attained economic importance on Long Island, nor elsewhere in the State. The highest proportion of diseased plants in the field never went beyond 10 percent, and this figure represented late infections in the growing season. Clayton indicated that rutabaga mosaic will never be of economic importance on Long Island, because the crucifers are grown when the temperature is relatively low and unsuited for development of the disease. He further said that, because of the usually mild winters on Long Island and of the fact that brussel

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[3] Numbers in parentheses refer to Literature Cited, p. 125.

sprouts (*B. oleracea* L. *gemmifera* DC) and cabbage are both relatively winter-hardy, they are the cruciferous suscepts that serve as primary sources of inocula for the next growing season.

The purpose of this paper is to present the data collected in regard to symptoms, suscept range, incubation period, and physical properties of a virus responsible for the mosaic of kale, and to express an opinion on the nature of the viral entity in agreement with recorded observations.

REVIEW OF LITERATURE

Cruciferous viruses tend to fall into two marked categories (11); one represented by the turnip virus 1 Hoggan and Johnson, including the cabbage virus A (22), the cabbage black ring virus (4), the cabbage ring necrosis virus (8), turnip virus described by Tompkins (19), Hoggan and Johnson's turnip virus (7), turnip virus described by LeBeau and Walker (9), and perhaps many others; the second represented by the cauliflower virus 1 Tompkins, which includes cabbage virus B (22), the cauliflower mosaic virus (18), Tompkin's Chinese cabbage virus (21), and the broccoli virus described by Caldwell and Prentice (1).

The first publication on a transmissible virus of cruciferous suscepts was made in 1921 when Gardner and Kendrick (3) obtained mechanical transmission of a virus from a mosaic-affected turnip to other crucifers, but not to radish (*Raphanus sativus* L). Schultz (13) using mechanical means and the vector *Myzuz persicae* (Sulz.) successfully transmitted from suscept to suscept a virus causing mosaic in turnip (*B. rapa* L.), Chinese cabbage (*B. pekinensis* (Lour) (Gagn), and mustard (*B. japonica* (Thumb) Sieb). Chinese cabbage mosaic has been reported from many parts of the world. Tompkins and Thomas (21), reported the disease from California and did extensive work on it. Turnip and rutabaga mosaic also is widespread and has been the object of studies by Tompkins (19). Reports of mosaic on *Raphanus sativus* caudatus (L) Bailey and on charlock (*B. arvenis*) have been made from India and Denmark (5). Noble (10) reported a cauliflower mosaic from New South Wales. He stated that it differs from the cauliflower mosaic reported by Tompkins from California. Hino (6) from Japan listed cruciferous suscepts of mosaic and mosaiclike diseases. Smith (14) reported a ringspot of cabbage from Cambridge, England, suggesting a similarity of this virus and the California virus.

In 1934 Tompkins transferred a cauliflower mosaic virus to cabbage. Similar results were obtained by Hoggan and Johnson (7) producing infections on cabbage with virus extracts from either turnips or horseradish and were the first to determine the properties of these viruses.

Tompkins (18) in 1937 reported a mosaic disease occurring on cauliflower in the cool, coastal valleys of California. In 1938 Tompkins, Gardner and

Thomas (20) described the black ring disease of cabbage, and indicated its common occurrence during the summer. Larson and Walker (8) described a ring necrosis of cabbage in Wisconsin. The presence of more than one virus entity was shown; virus A and B were found systemically in infected cabbage plants.

McWhorter (1936) reported a mosaic disease of crucifers, especially cabbage and cauliflower, in Oregon. This seems to be the same one that occurs in California.

MATERIALS AND METHODS

Plants used in the course of the work were grown in 4-inch clay pots or in flats, and kept at all times in an insect-free greenhouse at a temperature of approximately 22°C. The soil was sterilized with steam in all occasions. All material for inoculation such as cheesecloth, mortars and pestles, carborundum, test tubes, etc., were made aseptic by sterilization in the autoclave or in the Arnold sterilizer. All precautions to avoid contaminations were exercised.

Studies in the host range, the symptomatology and physical properties of the virus under consideration were conducted in the same greenhouse where the plants had been grown. Different trials were made in various compartments in the greenhouse at different air temperatures. To stimulate sap movement and new leaf formation in the plants to be inoculated, all excess leaves were removed and in some plants the process was to take away alternate leaves, or pinching off the terminal bud to promote suckering.

Mechanical inoculations were made by rubbing the inoculum with cheesecloth and with carborundum. The latter was sprayed over the leaves previous to inoculation. All plants that had not reacted to inoculation within a reasonable time were discarded. Cruciferous and noncruciferous plants with two or three pairs of leaves were inoculated and observed for virus-suscept reactions. In the case of noncrucifers, if any symptoms were detected, testing for infection was corroborated by taking juice from such plants and inoculating known cruciferous suscepts.

The physical properties of the virus were determined by treating viral extract from kale in the following manner: The inoculum was allowed to stand for a few minutes at room temperature (24°C), until the gross particles in suspension were precipitated. The supernatant liquid was removed with pipettes and immediately used to determine tolerance to dilutions and the thermal inactivation point. Part of the extract was kept in test tubes for the ageing trials. For tolerance to dilution, one cubic centimeter of the crude, decanted liquid was diluted with 9 cubic centimeters of sterile, distilled water; further dilutions being made to obtain various ratios.

In determining the thermal inactivation point, the clarified crude extract was distributed in serological, sterile tubes to about two-thirds of their capacity. Duplicates of these tubes were then treated at the desired temperatures in a thermostatically controlled water bath. For ageing, the crude, clarified extract was kept at 22°C. One tube was used each time for every required temperature.

EXPERIMENTAL RESULTS IN THE GREENHOUSE

Young crucifers and noncrucifers, with at least one pair of well developed leaves, were inoculated mechanically with infectious extracts from diseased kale. The technique described under the topic on methods was used. All crucifers were found susceptible to infection with the virus, the symptoms appearing within 10 to 20 days after inoculation. The lapse of time depended, apparently, upon the air temperature of the greenhouse and the type of plant or species utilized. The mosaic symptoms characteristic of the kale mosaic were reproduced in many of the crucifers; in others only some of the symptoms were evident. In some suscepts like rutabagas and kale, systemic mottling and chlorotic ring spots were reproduced while in cabbage and others vein-clearing, vein-banding, or mottling developed.

The data recorded strongly point toward the importance of air temperature, suscept species, and symptom expression, as well as to other events in connection with the incubation period. The crucifers reacted to inoculation within 10 to 14 days, if kept under an air temperature around 20° and took up to 20 days in a range from 14° to 17°C. No symptoms were noticed at temperatures either under 14° or above 30°C. Plants showing mosaic symptoms were taken to an environment with a temperature of less than 14°C., and the result was masking of the symptoms. When brought back to higher temperatures, the original symptoms reappeared.

In a study concerning the differentiation of crucifer viruses, Pound and Walker (11) determined that the cauliflower mosaic virus produced infection on the Jersey Queen cabbage seedlings in 18 to 21 days at a temperature of 16°C., in 14 to 17 days at 20°C., in 10 to 12 days at 24°C., and in 9 to 10 days at 28°C. These results indicate that the cauliflower mosaic virus from California and the kale mosaic virus from New York multiply more rapidly at the relatively higher temperatures. These workers have pointed out that cabbage virus B, the cauliflower mosaic virus, the Chinese cabbage mosaic virus, and the broccoli mosaic virus comprise a group confined to the Cruciferae, except for the Chinese mosaic virus which infects *Nicotiana glutinosa*. According to their work, virus B and the cauliflower mosaic viruses are related strains. It should be remembered, however, that virus B is cauliflower mosaic virus 1 category.

It has been shown by several workers (11, 12) that the severity of symptoms in infected plants varies with the species of the suscept and with the air temperature. Pound and Walker in Wisconsin noticed that mosaic-infected cabbage plants reacted with initial vein-clearing, vein-banding, and coarse mottling, and that as the temperature increased, the first two symptoms gradually disappeared, only mottling being present. When the air temperature decreased the first two symptoms again developed. They found that the Jersey Queen cabbage, when inoculated with cabbage virus A or B, reacted with severe symptoms at a temperature range from 16 to 20°C but the plants stayed symptomless at temperatures from 24 to 28°C. Indian mustard (*B. juncea*) showed mild spotting when inoculated with cabbage virus A at 20°C. Inoculation with virus B caused severe spot necrosis. *Nicotiana rustica* infected with virus A at 16°C. showed mild necrosis; with virus B severe necrosis.

In studying the mosaic disease of cauliflower Tompkins (18) found that pronounced symptoms were produced in this plant from 10 to 19° C. and that the symptoms disappeared in a temperature range from 20° to 30°C.

In determining the effect of air temperature and virus concentration Pound and Walker (11) found that in cabbage, virus A and black ring-spot virus multiplied rapidly at 28°, but very little at 16°C. Plants kept at 5°C. for 60 days showed no virus multiplication, but the titer became normal after the plants were brought back to 28°C.

The negative response to inoculation of noncrucifers, shows that the kale virus under consideration does not belong to turnip virus 1 category.

In table 1 it can be seen that *Beta vulgaris* reacted with pronounced anthocyanescence and small spot necrosis. However, the controls reacted in a similar manner. In order to clear this point, extract from beets was inoculated to cruciferous suscepts but no infection was obtained. Perhaps it is difficult to obtain the virus from beets in this way. If we examine the symptomatology and the extent of the suscept range of the viruses in the second category, it will be seen that the kale virus compares very closely with the cauliflower mosaic virus from California described by Tompkins in 1937. This investigator found that repeated inoculations with the cauliflower mosaic virus failed to infect *Nicotiana glutinosa*, *N. tabacum*, and *N. langsdorffii*. All crucifers were found susceptible and, so far, there are no suscepts from other families than the *Cruciferae*.

The possibility that the turnip virus from New York and the rutabaga virus described by Clayton (2) are identical, has been suggested (18). Tompkins found that the turnip virus causes infection on *Nicotiana tabacum*, *N. glutinosa*, rutabaga, and Pet-sai, and annual stock, but no infection was obtained on sprouting broccoli, kohlrabi, radish and *N. langdorsffii*, showing that the turnip virus and the cauliflower virus are

TABLE 1.—*Reaction of different species of cruciferous and noncruciferous plants to artificial inoculation with infectious extract obtained from kale mosaic infected plants*[1]

Family species and common name	Symptoms—	
	From 14 to 18°C.	From 20 to 27°C.
Brassica oleracea capitata L. (cabbage var Cape)	Symptoms similar to those above or marked	Light mottling, almost inconspicuous, no distortion of leaves, no necrosis
B. oleracea L. *gemmifera* DC. (brussel sprouts v. Long Island Improved)	No leaf malformation, no necrosis	Vein-clearing, later mottle
B. oleracea botrytis L. (cauliflower v. Snow Ball	Similar symptoms as for preceding species	Vein-clearing, faint mottle, no necrosis
B. oleracea gongylodes (kohl-rabi v. Early White Vienna)	Similar to above, but symptoms appeared somewhat later	Vein-clearing and systemic mottling with no leaf distortion and no necrosis
B. oleracea L. var *acephala* (kale v.)	Similar symptoms, but lighter mottling	Vein-clearing and systemic mottling, chlorotic rings, no necrosis, no distortion of leaves
B. campestris L. *napobrassica* DC. (rutabaga v. American Purple Top)	Similar symptoms as for above species	Vein-clearing and systemic mottling, chlorotic rings, no malformation, no necrosis, rings disappearing in older leaves
Raphanus sativus L. (radish v.)	Similar results as for above species	Light mottling as small chlorotic spots, no necrosis
B. rapa (L) Bois (turnip v. Flat Purple Top)	Similar results to the ones obtained with preceding species	Light mottlings as small chlorotic spot, no necrosis
B. pe-tsai Bailey (Chinese cabbage v.)	Light mottle	Vein-clearing, followed by mottling, distortion, and curvature of the midrib, stunting of plant, at high temperatures chlorotic rings and no necrosis

[1] Inoculations were made on March 7, 1949—not less than 5 plants of each species tested.

different entities and belong to different categories. Very little work has been done with the rutabaga virus and what was done did not include the study of the physical nature of the virus nor the immunological and serological reactions to compare with other similar viruses reported on crucifers.

So far the work done by Clayton (2) gives the only available description of this virus on rutabagas.

Noncruciferous, Resistant Species

The following species were tested and found not to become infected by the virus:

Nicotiana tabacum L. v. Turkish
N. glutinosa L.
N. langsdorffii Weinm.
N. rustica L.
Solanum melongena L. (American Black Beauty)
Lycopersicum esculentum Mill. (John Baer)
Datura stramonium L. (jimsonweed)
Solanum nigrum
S. integrifolium
Capsicum frutescens
Apium graveolens L. (celery)
Spinacia oleracea L.
Beta vulgaris L. (beets)
B. vulgaris var. cicla Moq. (swiss chard)
Lactuca sativa L. (lettuce v. Simpson)
Gomphrena globosa L. (bachelor's button)
Phaeseolus vulgaris L. (common bean)
Pisum stivum L. (garden pea)
Medicago sativa L. (alfalfa) (clover)
Vigna sinensis (black cowpeas)
Cucumis sativus L. (cucumber)
Zinnia elegans (zinnias)
Physalis sp.
Portullaca sp.

PROPERTIES OF THE VIRUS

In testing the physical properties of the Kale virus, infectious extract from diseased kale was applied with the help of carborundum to inoculate cruciferous suscepts. The results obtained showed that the virus has an approximately longevity in vitro of 13 to 14 days, at temperatures around 20°C. to 22°C. The virus was not inactivated at a temperature around 80°C. for 10 minutes. The tolerance to dilution was somewhere from 1:1800 to 1:2000. (See table 2.)

Tompkins (18) found that the cauliflower mosaic virus has an age in vitro of 14 to 15 days at 22°C., thermal inactivation was around 75°C, and its dilution tolerance is 1:2000. Others have reported a dilution tolerance up to 1:3000.

In accordance with the data obtained in the course of this exploratory work, it can be said that the behavior of the kale mosaic virus is very similar to that described by Tompkins (18) for the cauliflower mosaic virus from California. There is, however, an apparent disparity in the symptomatological reactions at different temperatures, the virus from kale reacting more definitely at temperatures around 20 to 22°C., while that of Tompkins ran from 10°C. to 19°C. This could perhaps be explained by the possibility that the entity from kale is a different virus, or a strain of the cauliflower mosaic virus.

TABLE 2.—*Properties of the kale virus as determined by mottling in a cruciferous suscept (Kohlrabi)*[1]

Longevity in vitro		Thermal death point		Tolerance to dilution	
Exposures at 22°C.	Reaction to inoculation	Temperature of the 10-minute exposures	Reaction to inoculation	Dilution rate	Reaction to inoculation
Days		°C.			
1	x	45	x	1:10	x
2	x	50	x	1:100	x
3	x	55	x	1:200	x
4	x	60	x	1:300	x
5	x	65	x	1:400	x
6	x	70	x	1:600	x
7	x	75	x	1:800	x
8	x	80	x	1:2000	x
9	x			1:1400	x
10	x			1:1600	x
11	x			1:1800	x
12	x			1:2000	—
13	x				
14	—				

[1] Not less than 3 plants per test.

In view of the limited amount of work done in determining the nature of the virus from kale, it cannot be stated definitely that the kale virus is the cauliflower mosaic virus. Further and more extensive work should be done in which the virus from the kale and the cauliflower are compared simultaneously under identical environment, the same species of suscept plants being used in both cases.

SUMMARY AND CONCLUSION

The results obtained from several pathogenicity trials with leaf extracts from a kale (*B. oleraceae* var. *acephala*) plant showing mottle and chlorotic

ring-spot symptoms, have disclosed that the physiological disturbance is produced by a virus.

The virus can readily be obtained from kale and transmitted mechanically with carborundum to several crucifers, but not to noncrucifers. The reaction of crucifers to inoculation is characterized by initial vein-clearing, vein-banding, systemic mottling, and chlorotic ring-spots on the leaves.

Among the crucifers tested, kohlrabi, cauliflower, Chinese cabbage, kale, and rutabaga showed the most conspicuous symptoms of mosaic. Kale, rutabagas, and Chinese cabbage developed chlorotic ring-spot symptoms and mottle; and Chinese cabbage reacted usually with faint mottle and leaf curling. The symptoms were seen clearly at temperatures around 20°C. and gradually became inconspicuous at temperatures below 14° or above 27°C.

Brussells sprouts and broccoli were slow in showing mottle symptoms and cabbage appeared to be the least to react to infections, very faint symptoms being produced at the optimum temperatures.

In comparing the cauliflower virus from California and the turnip virus from New York, Tompkins (18) found that the turnip virus produced symptoms in Colma cabbage and February cauliflower very similar to those described by Clayton (2) on brussels sprouts and cauliflower, but Tompkins demonstrated that turnip virus from New York caused symptoms on *Nicotiana tabacum* and *N. glutinosa*, brussels sprouts, rutabagas, and Chinese cabbage, and no infection on sprouting broccoli, kohlrabi, raddish, and *N. langdorsffii*, thus showing that the cauliflower mosaic virus is different from the turnip virus and the rutabaga virus described by Clayton from New York.

The work done in Cornell with the kale mosaic virus indicates that the kale mosaic virus and the rutabaga mosaic virus described by Clayton are related to the cauliflower mosaic virus described by Tompkins (18) from California. Clayton did not determine the physical properties of the rutabaga virus.

The virus from kale does not cause infection in noncrucifers; its physical properties are: Thermal inactivation point around 80°C., dilution tolerance of 1:2000, and aging in vitro approximately 14 days. All these strongly point to the conclusion expressed above.

An apparent discrepancy between the kale mosaic virus and that of the cauliflower rests on the temperature range for maximum symptom expression, though not for infection. Tompkins (18) has shown that the cauliflower mosaic virus produces the most marked symptoms in cauliflower at temperatures from 10°C., and that at temperatures from 20–30°C., the symptoms are masked. However, there is the possibility that, even

though there is masking of the symptoms, the rate of multiplication of the virus is not suppressed by higher temperatures, if we consider the results obtained by Pound and Walker (11) in which they showed that, at temperatures of 16°C., the cauliflower mosaic virus studied produced mottle symptoms in Jersey Queen cabbage in 18 to 21 days, while at higher temperatures the incubation period was gradually decreased, and at 28°C. the time for symptom expression was within 9 to 10 days. The titer of the virus at the different temperatures was not determined, therefore there is no evidence of the relation of time-of-incubation period and virus multiplication at various temperatures.

One explanation of our apparent discrepancy as to the epiphytology of this disease could be based on daily fluctuations of the temperatures in the greenhouse, which veils the true relations of the temperature and the incubation and symptom expression of the disease. One thing is obvious, and that is that the temperature ranges used in our work were very wide, and there were opportunities for symptoms to develop either when the temperature was at the lower or at the higher limit of the range, or at any point therein.

In order to have a clear picture of the situation it would be necessary to conduct further trials under constant air temperatures, running parallel tests with the cauliflower mosaic virus for comparative results.

In his work with the true cruciferous virus, Clayton found that rutabagas, white and black mustard, Chinese cabbage, turnip, and rape were susceptible; brussels sprouts and cauliflower not easily infected; and cabbage was either resistant or immune. These last results do not seem to be conclusive since Clayton did not use carborundum in his work. At 21 to 27°C. he found that Chinese cabbage and mustard developed streaks. At 12 to 18°C., brussels sprouts and cauliflower recovered completely and the symptoms disappeared either below 13°C. or above 27°C.

The difference between the true cauliflower and the cauliflower mosaic viruses is based on the virus-suscept reaction at different temperatures.

Tompkins' virus works best at temperatures from 10° to 19°C., and Clayton's at temperatures from 20° to 27°C.

The trials conducted here in connection with the kale mosaic virus are too limited to warrant the exact classification of the virus. They do, however, show that the kale mosaic virus belongs to the cauliflower virus category, and is very closely related to the cauliflower mosaic virus described by Tompkins from California. The kale mosaic virus is either the cauliflower mosaic virus or a very close virus entity. Further work on cross-immunity reactions and possibly serological tests could clarify the situation.

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A NEW DISEASE OF ORCHIDS IN PUERTO RICO: PYTHIUM BLACK ROT

L. A. ALVAREZ GARCÍA[1]

Reports of a black rot of orchids, (*Laelio—Cattleya* and *Cattleya* spp.) growing in greenhouses and outdoors in the Metropolitan Area, were received early in November 1949.

The weather was chilly and humid during the month of November and December 1949, and through February and March 1950. The temperature during November and December varied from a low of 16°C., during the night to a high of 26°C. during the day. At the end of February, 1950 temperatures from 20 to 24°C. at night, and 26 to 30°C. during the day were prevalent. The average relative humidity was around 90 percent most of the time because of frequent rains and foggy weather.

CHARACTERISTICS OF THE DISEASE

Symptoms: The disease is characterized by the development of black spots of variable sizes on pseudobulbs, leaves, stem, and roots. At first the lesions on the pseudobulbs, are recognized as dull green, minute spots. These enlarge and become light yellow as they grow older. The diseased areas finally turn brown or black. The lesions spread very rapidly especially on young, succulent pseudobulbs. The longitudinal rate of enlargement is from 2 to 3 inches per day, extending also around the pseudobulbs.

The infections are confined to the parenchyma tissue, and are characterized by the disorganization and decay of the cells without foul odors. The fibrovascular bundles remain entire, though they become dark and appear as fibers or threads within the rotted parenchyma tissues. The infected pseudobulbs rot in 12 to 14 days in tender pseudobulbs after the symptoms of infection are shown. In older pseudobulbs, the rate of development of the infection is delayed but eventually they decay completely.

The lesions start as small, dull spots or narrow streaks on the leaves. These lesions appear the margins and tips and other times scattered over the blade. Spots are frequently found on the midrib and at the base of the leaf. The enlargement of the disease areas on the leaves is not as rapid as in young pseudobulbus. Apparently, the higher moisture content of the pseudobulbs accounts for the differential growth rate of the pathogen. Diseased leaves are shed prematurely when the infections are produced at the base of the leaves, or at the tip of the pseudobulbs. When the environment is humid for days, the pseudobulbs and leaves become soft and rot.

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When the weather after infection stays dry the pseudobulbs and leaves become hard. Under humid conditions, stems and roots are attacked by the organism and turn black. Under wet conditions the infection extends from one pseudobulb to another by way of the stem and roots, or by spattered sporangia during rainy weather (fig. 1).

ETIOLOGY

The pathogen was studied and found to be a strain of *Pythium ultimum* Trow. Isolates of *P. ultimum* from various diseased *Cattleya*, *Laelio-cattleya*

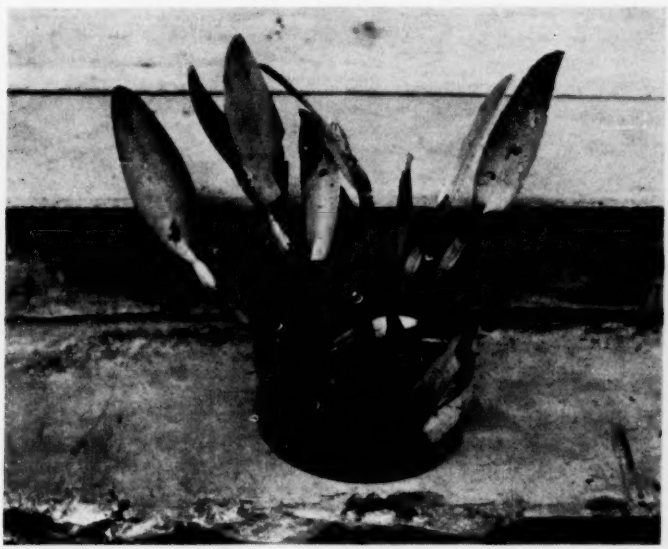

FIG. 1.—*Cattleya Gigas* infected with *Pythium ultimum*. Inoculation on axis of leaves.

and *Oncidium* showed the association of this fungus with the black rot. The isolates were obtained from infected material supplied by Mr. Ferdinand Méndez, Dr. Luis Fernández, and Mr. Ramón Aboy. Diseased pseudobulbs, leaves, stems, and roots were cleaned of extraneous material by repeated washings with distilled water. Pieces of diseased tissues were immersed in a 10-percent solution of sodium hypochlorite for 5 minutes. Some of these disinfected material was transferred and planted on culture media, in petri dishes. Other pieces were taken aseptically from the innermost tissue of flamed pseudobulbs and leaves and cultured in solid media in petri dishes.

The isolates grew readily in most sugary media and produced a super-

ficial white, radial growth, that covered the petri-dishes in a day or two after planting. The growth was most rapid at temperature from 26 to 30°C. The pH of the cornmeal culture media was 5.26.

In cornmeal agar the growth was white, light, and radial. The mycelium produced was thick and profusely knobed or gnarled. Sporangia were abundant, solitary, or in chains. The size of catenulated sporangia was variable, some were around 5 to 6 μ., others as large as 30 μ.; the shape was spherical or papillate. In other cases they were elongated and produced intercalary or at the tips of hyphae (fig. 2).

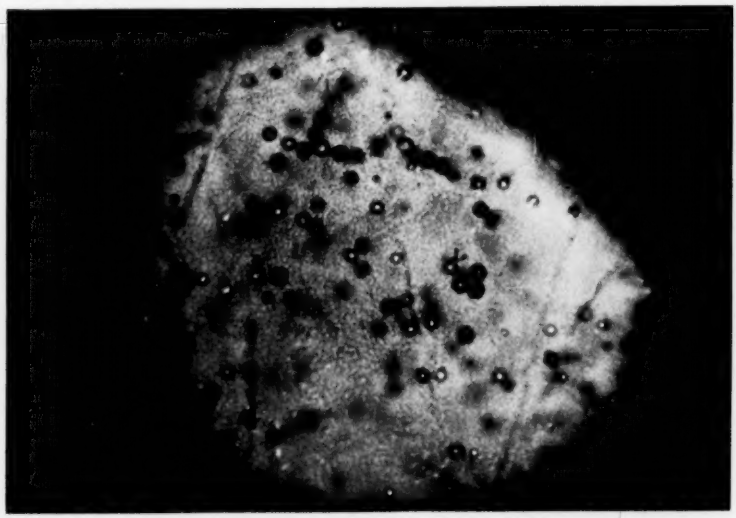

FIG. 2.—Microphotograph of *Pythium ultimum*, showing sporangia.

Large spherical sporangia were of varying sizes, measuring from 15 to 52 μ. in diameter. The sporangial wall was around 2.5 μ. The contents of the sporangia was brownish in color and the coenocentrum was large, subspherical, and refringent.

Oogonia were scanty or absent. They were similar to sporangia in shape and size as well as in other characteristics. They could be distinguished from sporangia only by the presence of antheridia. These are short, sessile, inflated, and upcurved and monoclinous. Germination of sporangia is by germ tubes. Germ-tube formation occur readily at ordinary room temperatures from 26 to 28°C., but the higher counts are at the lower temperature. Germination is observed at temperatures from 20 to 26°C. At temperatures above 28°C, very little or no germination occurred (fig. 3).

The organism is soil-harbored. It was found on the Station Grounds

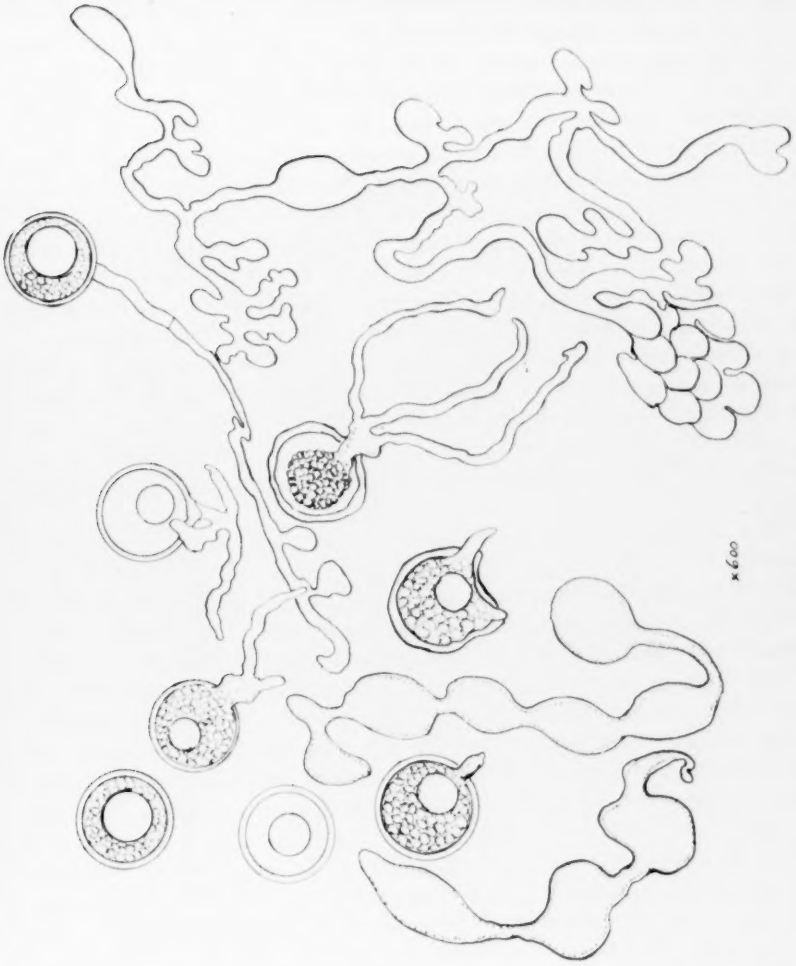

FIG. 3.—Pythium black rot of orchids.

causing damping-off of pepper seedlings in seed beds. The isolates from diseased pepper seedlings compared both in morphology and physiology with the isolates from *Cattleya*, *Laelio-cattleya*, and *Oncidium*.

Rosseti (1)[2] reported a black rot of *Laelia* sp. in Brasil, but she did not classify the organism, only mentioning that it was pythiaceous in nature. In this report a similar disease of *Cattleya* and *Vanda* from Puerto Rico, and *Stanhopea saccata* from an unknown place, is mentioned. Porter (2), in Puerto Rico, published a paper dealing with a black rot of *Cattleya* caused by a *Diplodia* sp. resembling *D. natalensis*. Artificial inoculation with the organism caused infection on *Lycaste skinneri*, *Epidendrum nodosum*, *Oncidium variegatum*, *O altissimum*, *Vanilla fragrans*, *V. barbellata*, *V. ecuadori*, *V. pompona*, slow development of the infection occurred on *Cyrtopodium punctatum*, *Laelia crispa*, *L. superbens*, *Catasetum* sp., *Gongora* sp., *Epidembrum lifidum*, *superbens*, *Catasetum* sp., *Gongora* sp., *Epidembrum lifidum*, *Lelopsis domingensis*.

Limber (3) determined the distribution of pythiaceous black rot of orchids based on interceptions from Venezuela, Colombia, Cuba and England.

In California, Ark and Middleton (4) described a black rot of *Cattleya* caused by a strain of *Pythium ultimum*. Sporangia and oospores measures 13.2–29.6 μ. in diameter, with an average of 21.9 μ. Infections are produced principally by zoospores. The optimal temperature for growth of the organism is about 28°C and for infection around 20°C. During isolation, a *Gloesporium* sp. and an organism resembling closely *Diplodia natalensis* were found. However, none of these organisms were capable of producing black rot of *Cattleya* even though they were inoculated by wounding the pseudobulbs and leaves. The inoculated plants were kept under a high relative humidity (90 percent) and at a temperature range of from 20° to 30°C. One thing was obvious, and that was that the *Gloesporium* and *Diplodia* species grew only following previous infection with *P. ultimum*. In regard to *D. natalensis*, it seems that Porter worked with another strain of the fungus. From his publication we cannot get information on the environmental conditions for infection.

EPIPHYTOLOGY

Cattleya kept in a glass cage, with a constant relative humidity of 90 percent, were inoculated with sporangia from a 15-day culture of *P. ultimum* growing in cornmeal agar with a pH 5.56. Five-milimeter pieces of agar of a 2-week culture on cornmeal agar with abundant sporangia, were placed on the surface of succulent pseudobulbs. In another test a porangial suspension was sprayed over young leaves. A drop of water

[2] Figures in parentheses refer to Literature Cited p. 135.

containing sporangia was placed also with a sterile dropper at the base of the leaves at the natural pocket formed with the pseudobulbs. Under these conditions infections were recognized in 5 to 7 days (fig. 4). Controls were similarly treated but this time sterile water used.

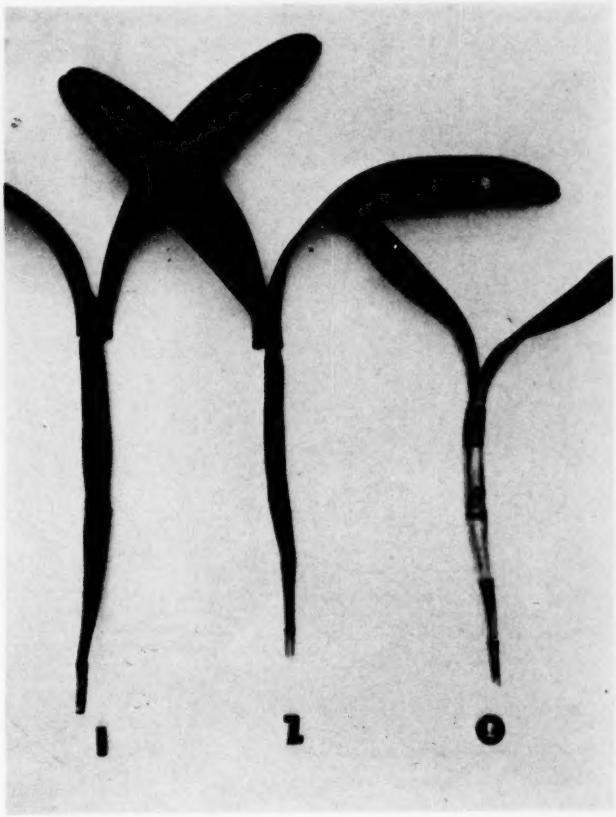

FIG. 4.—1 and 2 Black-rot lesions on pseudobulbs and leaves upon artificial inoculated with *Pythium ultimum* O– check.

The lesions were dull green at first, later became light yellow and gradually turned brownish or dark. Infections on the pseudobulbs spread rapidly, at the rate of 1 to 2 inches up and down the point of penetration of the fungus. The fate of development of the infection was particularly rapid in young, tender pseudobulbs. In a week, the pseudobulbs were completely rotten. On pseudobulbs with more wood-tissue, the rate of development of

the lesion was slow, and in very old pseudobulbs the lesion took over a month to spread. There is a close association of the degree of succulency of the pseudobulbs and the development of the lesions. (Figs. 5 and 6.)

On the leaves the infection was started as a small water-soaked, dark area which later turned dark (fig. 7). Twice the time was needed to rot

FIG. 5.—*Cattleya Bowringiana*. General appearance of an orchid plant recently inoculated with *Pythium ultimum*. Note several pseudobulbs with black-rot lesions.

the leaves when compared with tender pseudobulbs. Infections started at the base of the leaves, spread rapidly along the midribs, and took about 14 days to cover the leaf (fig. 8). Inoculation tests, following Koch's rules, showed the pathogenicity of the various isolates.

High humidity (80 to 90 percent) for at least 2 or 3 days, and temperatures of from 20° to 30°C were favorable for infection.

Cattleya placed in a greenhouse with a low relative humidity, (50 to 60

per cent) were difficult to infect, unless the pathogen was inoculated through wounds and even so, the infections were arrested from spreading to other pseudobulbs. Infections occurred rapidly in plants left outside and exposed to a high relative humidity. The organism spread by way of infected stem and roots from one pseudobulb to another.

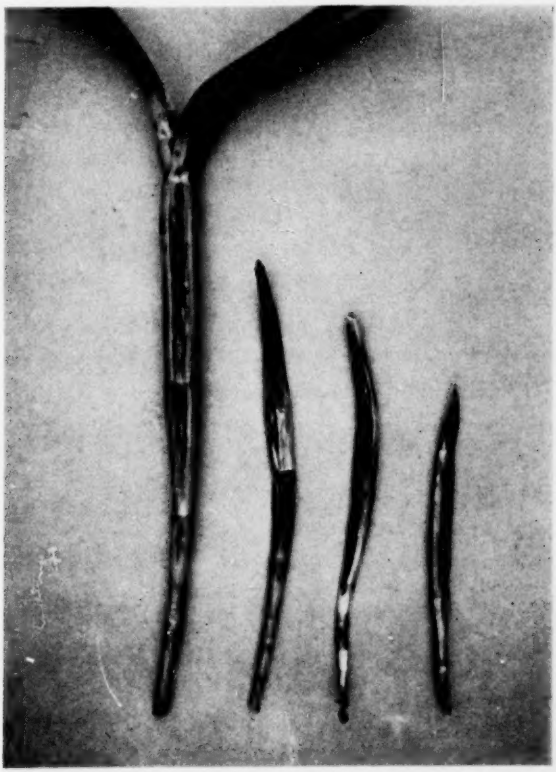

Fig. 6.—*Cattleya Bowringiana* artificially infected with *Pythium ultimum* isolated from *Cattleya*. Infected pseudobulbs.

In testing for resistance, the orchids *Cattleya Bowringiana, C. Skinnerii; C. Gigas; C. Mossiae; C. Percivaliana; C. Gaskeliana; C. Trianae* and *C. Dowiana Aurea* were found susceptible to the disease. The lesions on young pseudobulbs appeared in 2 or 3 days after inoculation. Infected pseudobulbs were rotten in 10 to 12 days. The lesions took about twice that time to rot the leaves.

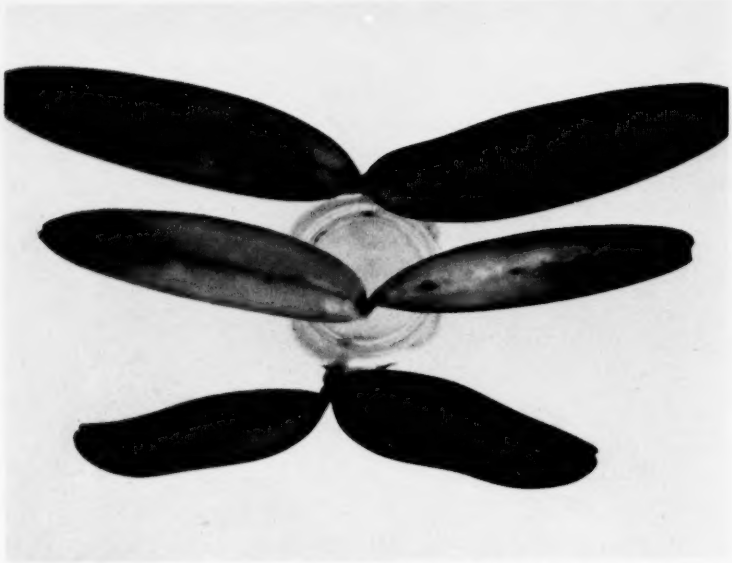

FIG. 7.—*Cattleya Bowringiana* inoculated with *Pythium ultimum*. Lower pair of leaves was not inoculated.

FIG. 8.—*Cattleya Bowringiana* inoculated with *Pythium ultimum*. Note development of lesion from the base of the leaf up.

CONTROL

In diseased plants, infected pseudobulbs were removed. The plants were then treated by spraying with Parzate and Dithane Z-78 (2 pounds in 100 gallons water) every other week. Other plants were sprayed with Bordeaux (2-3-50) and Copper A Compound (2 pounds in 100 gallons). Some of the sprayed plants were left outdoors and no further symptoms of the disease were observed for 8 months.

Other plants were similarly treated but sprayed with water. In this instance, the orchids were placed in greenhouse kept at a relative humidity of 50–60 percent. No indication of the disease was observed under this dry environment. Plants artificially infected but not treated, and left in the greenhouses showed no spreading of the infection to other pseudobulbs or leaves.

Infected plants kept outdoors in a high relative humidity (80 to 90 percent) succumbed to artificial infections.

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THE FOOD OF *RANA CATESBEIANA* SHAW IN PUERTO RICO

MARIO E. PÉREZ[1]

HISTORICAL NOTES[2]

The common bullfrog, *Rana catesbeiana* Shaw, of the Southeastern United States was introduced to Puerto Rico in 1935 by the Insular Department of Agriculture and Commerce. Mr. Adger C. Smyth, head of the Ornithology and Pisciculture Service, notes in its 1935 Annual Report that a total of 40 frogs from Florida were placed in a specially constructed pond at Río Piedras. In the 1936 Annual Report he stated that the introduction and breeding of the bullfrog in Puerto Rico had been very successful.

The present range of the bullfrog in the Island is not known exactly, but may be expanding rapidly in the more humid coastal areas. The frog is at present definitely known to be in the environs of Río Piedras and neighboring towns, and at Mayagüez and Humacao. Most recently it was reported from Barceloneta, where it caused a great deal of nervousness among the inhabitants who had never before heard the noise made by the male frog. So many stories and superstitions were current about these noises in the night that the Station had to explain the cause in the newspapers to bring peace to those people. The same thing happened some 10 years ago in the vicinity of Río Piedras, but this time a continental lady, Miss Asca Watson, who probably had heard the bullfrog's croak many times in the States, cleared up the mystery. She called a group of neighbors one night and with the aid of a flashlight and a home-made net caught the amphibian ghost and showed it to the astonished people.

REASONS FOR UNDERTAKING THIS WORK

The leading crop in the island of Puerto Rico is sugarcane, the cultivation and manufacture of which employs around 40 percent of the Island population. Other crops are pineapples in the coastal areas and coffee and tobacco in the mountains.

Together with the ravages caused by the sugarcane mosaic disease around 1915–1920, white grubs of the genus *Phyllophaga* have most seriously threatened the sugarcane industry. They attacked not only sugarcane but almost every other crop grown. Mr. Harold E. Box, Entomologist

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[2] Presented at the sixty-first Annual Meeting of the American Association of Economic Entomologists, at Tampa, Fla., Dec. 16, 1949.

of Central Aguirre (1925),[3] gave a good picture of the seriousness of the situation when he noted that "the administration of one of the largest concerns operating in the south coast of Puerto Rico spent approximately $15,000 upon six million grubs and 4 million adults of *Phyllophaga* during five years 1919–1923 inclusive, in hand collection control".

The situation had become so critical that, with the objective of finding a remedy for this pest, the Sugar Producers Association of Puerto Rico founded the Agricultural Experiment Station now at Río Piedras.

Mr. D. W. May, Director of the Federal Experiment Station at Mayagüez, introduced a few toads, *Bufo marinus* (L.), from Barbados; in 1920 and 1923, Mr. R. Menéndez Ramos, Director of the Insular Experiment Station, made an introduction from Jamaica of the same species of toads, which were released at Río Piedras.

The studies by Mrs. Raquel Dexter (1932), showed that no factor other than the toad had contributed more to the rescue of the main agricultural crop from destruction by white grubs.

However, the hero of such a wonderful performance was readily forgotten by many a farmer, as well as by the large sugarcane corporations, until recently the toad population began to decrease. Among the factors responsible are: (1) the prolonged periods of drought that dried up the most important breeding areas, especially the Cartagena and Guánica lagoons in southwestern Puerto Rico, (2) the abundance of the aquatic larvae of the Dystiscid beetle, *Megadytes giganteus* Castelnau, predaceous on the immature stages of the toad, (3) lack of food after most of the Maybeetle adults of the white grubs had been devoured by the toads and (4) ignorance of farmers who destroyed the toads because they thought that they ate chicks.

It was thought that one of the additional factors possibly affecting the reduction of the toad in Puerto Rico might be competition with the increasingly abundant bullfrog. The adult frog, living in the same environment as the toad's pollywogs, might also be eating them, for there was evidence that the frog in the States ate frog pollywogs (Frost, 1932). With the help of Dr. George N. Wolcott, and at his suggestion, the author started studying the stomach contents of the bullfrog in Puerto Rico.

METHODS OF COLLECTION AND STUDY

With the aid of a long-handled net the frogs were caught as early in the morning as possible in a nearby brook.

Mr. Harold C. Plank, Entomologist for the Federal Experiment Station in Puerto Rico, very kindly sent 12 alcoholic-preserved alimentary tracts from the Mayagüez area.

[3] For this and other references see Literature Cited, pp. 141–42.

A total of 50 frogs were examined during the year 1948–49, starting with the month of August.

Immediately after killing, the animal was dissected, the stomach contents placed in a petri-dish and examined with the aid of a binocular. The insects that were found were checked with the Station collection and the snails and undetermined insects were sent to the U. S. National Museum for determination. We are greatly indebted to Mr. C. F. W. Muesebeck for insect and snail identifications and to Miss Doris Cochran for confirming identification of the frogs.

FOOD ITEMS FOUND IN THE STOMACHS OF 50 BULLFROGS, *RANA CATESBEIANA* SHAW, IN PUERTO RICO

	Percent
Platyhelminthes	0.7
Trematode (free living species)	
Nematode	
Mollusca	3.7
Subulina octona Brugiere, determined by Div. of Mollusks,U. S. N. M.	
Australorbis glabratus (Say)	
Physa cubensis Pfeiffer	
Family Acteonidae	
Family Pupillidae	
Annelida	.3
Isopoda	.24
Sowbugs	
Arachnida	2.86
Cyrtopholis portoricae (Chamberlin)	
Family Marpissinae	
Red spider	
Myriapoda	5.52
Orthomorpha coarctata (Saussure), determined by H. F. Loomis	
Trigonulus lumbricinus (Gerstaecker), determined by Prof. Nathan Banks	
Unidentifiable Diplopod	
Dermaptera	.92
Doru albipes F.	
Anisolabis sp.	
Orthoptera	1.9
Periplaneta australasiae F.	
Supella supellictum Serville	
Pycnoscelus surinamensis L.	
Achetz assimilis F.	
Orocharis vaginalis Saussure	
Isoptera	.19
Nasusitermes costalis Holmgren, all soldiers	
Odonata	1.3
Enallagna sp.	

	Percent
Homoptera	.09
Agallia sp.	
Sibovia coffeaphila Dozier	
Undetermined fulgorids	
Undetermined aphids	
Hemiptera	7.00
Belostoma boscii Lep. & Serv.	
Limnogonus franciscanus Stål	
Undetermined coreid	
Undetermined lygaeid	
Coleoptera	3.94
Calosoma sp.	
Harpalus sp.	
Megadytes giganteus Castelnau	
Stetoxus intermedius Jacq. Duvál	
Dactylosternum sp.	
Parachalepus barbatus F.	
Selenophorus sp.	
Undeterminable Dytiscids	
Undeterminable Tenebrionids	
Anchonus suillus F.	
Metamasius hemipterus L.	
Cosmopolites sordidus Germar	
Diaprepes abbreviatus L.	
Diptera	.56
Asilid larvae	
Drosophilid fly	
Phorid fly	
Musca domestica L.	
Lepidoptera	.89
Prodenia sp. larva	
Pyralid caterpillar	
Acrolophus sp. larva	
Hymenoptera	2.14
Odontomachus haematoda L.	
Camponotus ustus Forel	
Solenopsis geminata F.	
Prenolepis longicornis Latreille	
Phleidole sp.	
Monomorium sp.	
Crassimicrodus sp.	
Apis mellifera L.	
Amphibia	3.90
Rana catesbeiana Shaw (pollywogs and small adults)	
Plant matter	10.58
Spathodea campanulata (seeds)	
St. Augustine grass leaves	

	Percent
Chilean willow leaves	
Clover leaves	
Cladophora moss	
Undetermined	3.36
Digested matter	45.10

DISCUSSION OF RESULTS

In none of the 50 frogs dissected during all seasons of the year in Puerto Rico was there any evidence that this Amphibian feeds on the toad's pollywogs. However, it ate pollywogs and small adults of its own species in Puerto Rico, as has previously been reported in the States (Frost, 1932).

Although the bullfrog is not as highly effective as the toad in devouring the most important economic pests of the island, it does eat them to a certain extent. Among the insects of economic importance found in the frog's stomach contents are the following:

Periplaneta australasiae—the Australian roach
Achetz assimilis—the common cricket, very destructive to foodstuffs, plants, and clothing.
Nasusitermis costalis—the "comején" termite
Metamasius hemipterus—the rotten cane-stalk borer
Cozmopolites sordidus—the banana corm borer, very injurious to bananas
Diaprepes abbreviatus—a common injurious leaf weevil which attacks almost every crop both as larva and adult
Megadytes giganteus—ferocious enemy of pollywogs
Prodenia caterpillars—cutworms
Pyralids (caterpillars)
Musca domestica—the common housefly
Solenopsis geminata—the fire ant, very injurious to many crops

It is rather interesting to note, comparing the food habits of the giant Surinam toad, *Bufo marinus*, with those of the bullfrog that the latter are considerably more diversified. According to Mrs. Raquel Dexter (1932) the highest total (43.3 percent) of the stomach contents of the toad was of Scarabaeid beetles, with very small percentages of other insects found. The bullfrog, on the other hand, shows more nearly equal percentages of the most important groups of insects and other small animals.

This difference in food eaten is most probably due to the fact that the bullfrog gets its food both from the water and from the ground, whereas the toad catches its food on the ground only.

The mollusk *Australorbis glabratus* (Say), the intermediate host of the common tropical disease known as Bilarhzia, has been found in a tenth of the frog's stomachs. Hoffman and Janer (1941), found that in a series of

analysis of feces of the toad *Bufo marinus* (L.), 26 had the parasite's ovum alive. Dr. Hoffman considered the toad as a potential menace acting as a mechanical vector of the disease. Having in mind that the frogs' habits are more restricted to water, we might expect the frog to be an even greater menace as a mechanical vector of the parasite.

The predacious larvae of *Megadytes giganteus* devours with insatiable appetite the pollywogs of both frog and toad, as well as other aquatic insects, including its own smaller larvae. However, the adult frog avenges this by eating the adults of the beetle.

Another instance of natural retribution is also shown in the food habits of the frog feeding frequently on the common Puerto Rican water bug, *Belostoma boscii* Lep & Serv. This aquatic hemipteran and other species of the group, have been reported as feeding on fish, as well as pollywogs and aquatic insects. They are strongly attracted to light in such great numbers that in many instances they are a great nuisance. L. O. Howard (1908) states that in Washington they fly to electric lights close to fish ponds, later falling to the water and devouring the fishes. Presumably, in Puerto Rico they feed on beneficial fishes like *Gambusia* spp. introduced for mosquito control.

In general, the bullfrog eats the same kinds of food in Puerto Rico as has been reported in continental United States (Frost, 1924), practically no items being beneficial to man, and although the majority are neutral, some are injurious species. The introduced bullfrog by no means eats as many injurious insects as does the introduced toad, and indeed, eats so few of them that it can hardly be considered a serious competitor with *Bufo marinus*.

The adult of each inhabits its own niche, and if the pollywogs of both live in the same pool, there is always an ample supply of ever-renewed algae to feed them. Nor is the bullfrog predaceous on either the pollywogs of the toad, or the very small adults, and any decrease in the abundance of the toad in Puerto Rico must be ascribed to other factors than the introduction of the bullfrog.

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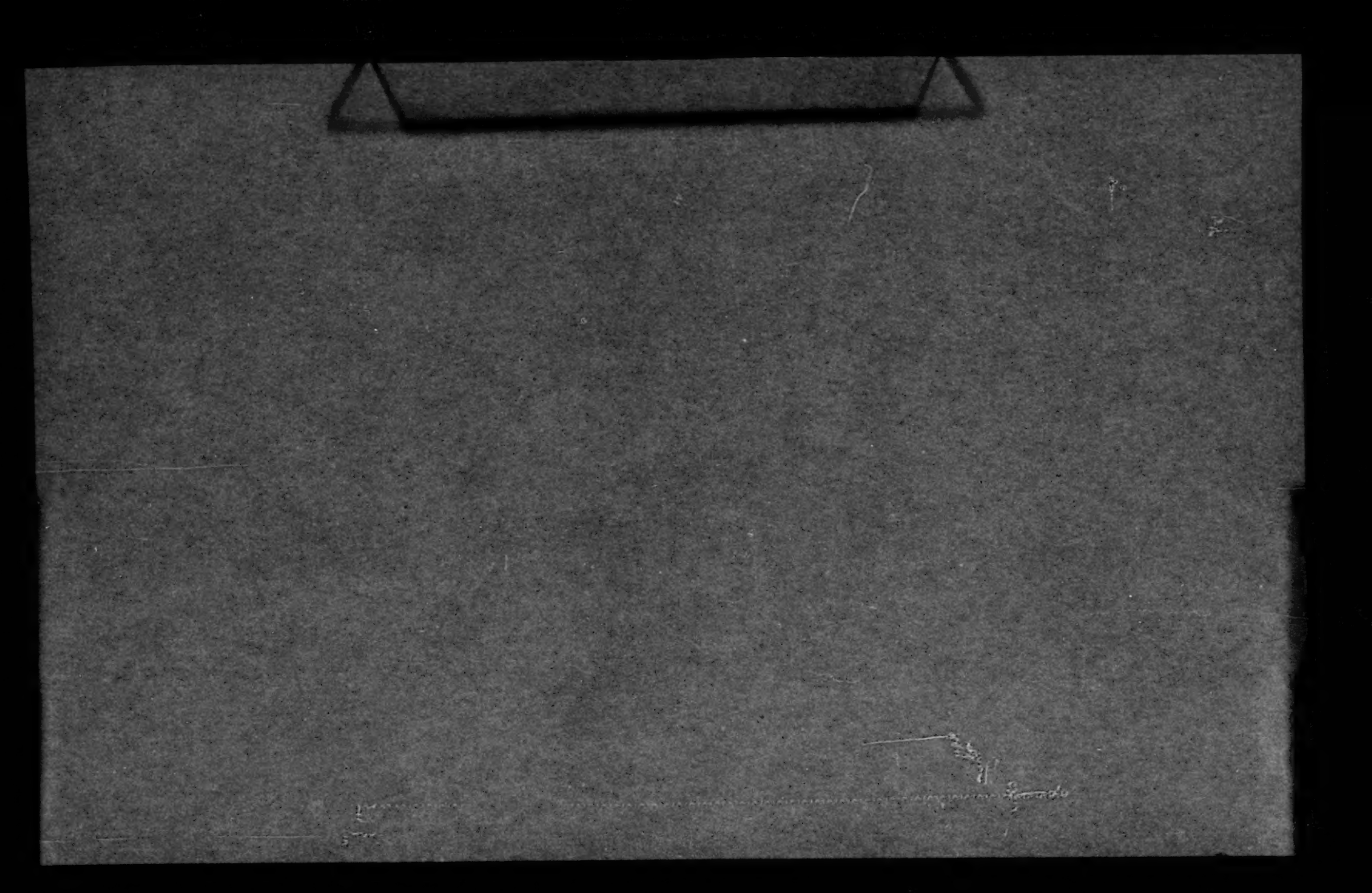

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